Evaluation of *Chlorella* as a Potential Drug for Nuclear Disaster Management

Synopsis

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1. Introduction:

There has been a very sharp increase in the use of radioactivity during past five decades, for both the well being and destruction of human beings. Radioisotopes like ^{99m}Tc, ^{67}Ga, ^{111}In, ^{130}I, ^{125}I, ^{57}Co, ^{51}Cr, ^{32}P, ^{59}Fe and ^{133}X have been used for therapy and diagnosis of various diseases (Garland and Heald 1957; Smithers 1958). Radioactive contamination consists of radionuclides that emit high-energy alpha, beta, and/or photon gamma radiation. A nuclear disaster during military, industrial, or medical practices leads to release of radionuclides which results in external and/or internal contamination with radioactive materials. External contamination is associated with the short-term effects of radiations. It includes the contamination of skin surface, foodstuff, water and gamma radiations from the spread of radioisotopes in the atmosphere. About 95% decontamination can be achieved by removal of clothing, and washing with soap or detergent and water. Whereas, internal contamination occurs when radioactive materials have ingested, inhaled, or absorbed from a contaminated wound. Once inside the body, radionuclides pose major health problems due to their emission characteristics in the form of alpha, beta particles or gamma rays. The only effective method of reducing these risks is removal of radioactive contaminants from the body. A number of important factors like absorption, excretion, concentration, chemical nature and effective half-life must understood for the removal of radionuclides from the body (Testa and Shields 1981; Wood et al. 2000).

Decorporating agent is a broad term that includes all agents that can either prevent the incorporation of the radionuclides or decorporate the incorporated radionuclides. These agents are categorized as blocking agents, chelating agents, diluting agents and ion exchanger. Blocking agents can enhance elimination or decrease incorporation of radionuclide. After incorporation, chelating agents, that can mobilize the radionuclides, are much less effective. It should be obvious that the lesser incorporation will occur with early administration of the proper decorporating agent. Chelating agents bind metal ions into complexes, thus preventing tissue

uptake and facilitate urinary excretion. Dilution of the radionuclide involves administering large amounts of a stable nuclide so the hazardous radionuclide has diluted. Thus, the decorporating agents can be used at three different levels in the body:

1. Prevention of radionuclides absorption from g.i.t to systemic circulation.
2. Hastening the elimination of absorbed radionuclides from blood stream.
3. Decorporation of radionuclides from their target organs.

At present, Prussian blue (Radiogardase-Cs® Capsule) is the most widely used decorporating agent for radioactive and non-radioactive thallium and cesium that has been evaluated clinically (Pau 2000; Dunstana et al. 1994**;** Thompson and Callen 2004; Altagracia-Martínez et al. 2012). Apart from Prussian blue, several chelating agents like potassium iodide, Ca-DTPA and Zn-DTPA approved by USFDA and other regulatory bodies are used for removal of radionuclide. (Ansari 2004). Prussian blue (500 mg capsules), Ca-DTPA (0.5 gm vials), ZnDTPA (0.5 gm vials), Ca gluconate (20%, 10 ml vials), Na alginate (10 gm powder in 30 cc vial, add water to drink), D-penicillamine (250 mg capsules), ammonium chloride (500 mg capsules), Na bicarbonate (8.9%, 100 or 200 cc vials for intravenous use), dimercaprol (British anti-lewisite, or BAL, 300 mg/vial) and aluminium phosphate have been recommended to be used in nuclear emergencies (Chin 2007; Robinson 1981).

Most of the above-mentioned agent/drugs need medical supervision for their use in removing internalized radionuclides. However, in an accidental scenario where a huge population gets affected raises an emergency where the subject needs immediate medical care. Removal of radionuclide metal ion/s from a subject's body is very difficult to achieve therefore, more emphasis should be paid to prevent the internalization of radioactive metal ions (Chin 2007). The agents having high therapeutic index and feasible to give without medical supervision, may offer an excellent option to provide immediate medical care.

Nutraceuticals, being an excellent source of basic nutrition with high safety offer potential agents for its use in mass population for pre-hospital care situations. (Nasri 2014). In general, nutraceuticals include antioxidants, dietary supplements, fortified dairy products, citrus fruits, vitamins, minerals, herbals, milk, and cereals. (Lee 2017). *Chlorella* in pulverized (broken cell wall) is one such potential nutraceuticals, which has been reported extensively for its metal ion binding efficiency. The green alga, *Chlorella* is reported as biosorbent for various metal ions. It is highly effective in accumulating radionuclide like strontium, cobalt and thallium, and toxic metals like lead, cadmium, chromium, mercury, zinc etc. it has excellent capability to accumulate toxic metals and radionuclides intracellularly from the external environment. In this approach, the present study will envisaged to screen the binding and removal efficacy of pulverized *Chlorella* for radionuclide and/or heavy metal ions.

2. Review of Literature

2.1 *Chlorella*

A Dutch researcher, Martinus Willem Beijerinck in 1890 first discovered microalga *Chlorella,* having a well-defined nucleus (Beijerinck 1890). The name of the *Chlorella* is derived from two words '*chloros*' (greek) and '*ella*' (latin) means green and microscopic size, respectively. It is unicellular freshwater green algae with diameter of about 3 to 8 micrometers and belonging to the family *Chlorella*ceae. *Chlorella* in dried form consists of about 40-45% protein, 15-20% lipids, 20-25% carbohydrate, 5% fibre, and 10% minerals and vitamins (Belasco 1997). *Chlorella* is being used worldwide as a dietary supplement (Fradique et al. 2010; Li et al. 2002), colourant after carotenogenesis, preservative (Rodriguez-Garcia and Guil-Guerrero 2008) and food emulsion (Fernandes et al. 2012). *Chlorella* is consumed as a dietary food supplement and available in the form of capsules, tablets, extracts and powder (Liang et al. 2004; Yamaguchi 1996). *Chlorella* has a thick cell wall (100–200 nm), which give mechanical and chemical

protection (Sydney et al. 2014). This outer cell wall is tough and indigestible by the human body, so the cell wall must be processed before use. A disruption technique must be employed to access the most of the micronutrients, contained within the cell wall (Safi et al. 2014). The *Chlorella* with pulvarised cell wall allows higher digestibility and provides up to twice the nutritional benefits than broken cell wall. The composition of pulvarised *Chlorella* (*C. vulgaris*) is summarized in (Shim et al. 2009).

Chlorella has reported as one of the bioadsorbent, which adsorbed the metal ions on their cell surfaces (Edris et al. 2014). Different species of *Chlorella* such as *Chlorella vulgaris* (Solisio et al. 2017; Inaba et al. 1980; Ting et al. 1889), *Chlorella pyrenoidosa* (Hart and Scaife 1977), *Chlorella kessleri* (Švadlenková et al. 2005), *Chlorella fusca* (Wehrheim and Wettern 1994; Loos and Meindl 1982) have shown high specificity towards different metal ions. The present review was envisaged to elaborate the efficiency of *Chlorella* in decorporation of radionuclide ions.

2.2 Potential of *Chlorella* for removal of radionuclide and heavy metal ions

Chlorella has been studies extensively for various radionuclides and metal ions like uranium, cobalt, cesium, strontium, thallium and mercury, cadmium, lead etc. This section elaborates important studies where *Chlorella* has been evaluated for its radionuclide or metal ion binding efficacy. Techniques used in removal, synthesis, characterization, mechanism and application perspective of *Chlorella* for radionuclide and/or heavy metals from water, wastewater and animal system by *Chlorella* are as follows.

2.2.1 Uranium

Uranium is used as an abundant source of energy but its release can cause hazardous effects to environment and human (ATSDR 2013). **Vogel et al. (2010)** reported the removal of uranium in a mineral medium by living and dead cells of *Chlorella vulgaris*. Algal cells removed most of the uranium from the solution at pH 4.4. The *C. vulgaris* has many functional groups that are

responsible for the binding of uranium (uranium-algae complex). In case of living cells, carboxylic and organic phosphate groups were involved in binding whereas in case of dead cells additional inorganic phosphates come into action during the uranium-*Chlorella* interaction. The hydroxyl (-OH) and carboxylate (-CO) functional group of polysaccharides in algal cell wall play an important role in U(VI)-algal complexation (Barkleit et al. 2008; Amini et al. 2013). **Nakajima et al. (1979)** suggested the physico-chemical interactions comprise the majority of total radionuclide uptake. In *Chlorella*, optimum pH for uranium uptake is 5 at which H^+ was exchange for radionuclide species and reduced by PO_4^{2-}, CO_3^{2-}, HCO_3^-. UO_2^{2+} and UO_2OH^+. For *Chlorella vulgaris,* the binding capacities of radionuclide are ordered as UO_2^{2+}>> Cu^{2+}>> Zn^{2+}≥Ba^{2+}, Mn^{2+}> Co^{2+}, Cd^{2+}> Ni^{2+}, Sr^{2+} (Gadd 1988).

2.2.2 Cesium and Strontium

The radioactive cesium (^{134}Cs and ^{137}Cs) produced during nuclear fission emits beta and gamma radiation and its exposure to human induces tissue damage and disruption of cellular function (ATSDR, 2004a). The radioactive strontium (^{90}Sr) produced in nuclear reactors or during the explosion of nuclear weapons are most hazardous of all the radioactive isotopes. Radioactive strontium may cause damage to the genetic material and lead to the formation of cancer (ATSDR 2004b).

Ogawa et al. (2016) suggested that *Chlorella* powder (Source: *Chlorella vulgaris*) did not reduce ^{137}Cs level significantly under any pH conditions. It indicates *Chlorella* did not have binding capacity to ^{137}Cs. Although, the in vitro binding efficacy of *Chlorella* for ^{90}Sr increases with increase in pH. In addition, pretreatment with *Chlorella* significantly prevents the accumulation of strontium in the bone and whole-body of mice. It reflects, *Chlorella* hasten the excretion of radioactive strontium from body.

2.2.3 Thallium

Thallium is one of the radionuclides used for the diagnostic purposes in nuclear medicine. Thallium after absorption to systemic circulation gets distributed in the kidneys (3%) and all other organs (97%). Thallium follows the movement of potassium and it excreted by the bile in enterohepatic recirculation. The fecal to urine excretion ratio of thallium is approximately 2:1 (Friberg et al. 1986; Lie et al. 1960).

Birungi and Chirwa (2015) studied the adsorption potential of various algal species; *Chlorella vulgaris, Scenedesmus acuminutus* and *Chlamydomonas reinhardtii* for thallium from eutrophic water sources. pH and contact time significantly affect the adsorption efficiency of *Chlorella* for thallium. The maximum sorption was around 95% after 24h of incubation at pH range 5-6. A monolayer adsorption of Tl on *Chlorella* results *in* high sorption capacity (1000 mg/g) and affinity (1.111 L/g) indicating *Chlorella* as a potential agent for removal of Tl. However, further investigations are needed to explore its effectiveness for removal of radioactive and non-radioactive Tl from living body.

2.2.4 Cobalt

Cobalt is naturally available in human body as a metal constituent of vitamin B12. However, radioactive Cobalt (^{60}Co) produced artificially in nuclear reactors and its leakage causes hazardous effects in nature. Human exposure to ^{60}Co causes numerous effects on health including cancers (Caravati et al. 2008; Leyssens et al. 2017). **Inaba et al. (1980)** reported the effect of *Chlorella vulgaris* on retention and tissue distribution of the radionuclide ^{54}Mn, ^{59}Fe, ^{60}Co and ^{65}Zn in rats. The whole body retention of ^{60}Co in rats treated with *Chlorella* was lower than the untreated group or control ($^{6o}CoCl_2$ only). **Geoffrey et al. (1992)** investigated the biosorption efficiency of *Chlorella salina* for cobalt ion from a solution containing 17 Bq mL^{-1} ^{6o}Co ($CoCl_2$ concentration 0.012 nM), 185 Bq mL^{-1} ^{54}Mn ($MnCl_2$ concentration 0.8 nM), and 50 Bq mL^{-1} ^{65}Zn (ZnC12 concentration 24 nM). *Chlorella salina* significantly adsorbed the cobalt ion at pH 8. The Bio-sorption of cobalt by *Chlorella salina* was pH dependent and also a

competitive interference was reported between Co^{2+} and other cations. **Švadlenková et al. (2005)** compared the uptake efficiency of ^{137}Cs and ^{60}Co by three microalgal species *Chlorella kessleri*, *Navicula atomus* and *Phaeodactylum tricornutum*. Metal uptake by these algal species varied significantly in case of both the radionuclides. *Chlorella kessleri* adsorbed about 5-10% for ^{137}Cs and 10 -50% for ^{60}Co.

2.2.5 Mercury

Ben-Bassat and Mayer (1977) compared the effect on uptake of mercury ($^{203}HgCl_2$) by previously exposed cells to Hg and normal grown cells of *Chlorella pyrenoidosa*. Both the cells showed identical uptake of Hg from medium. In addition, some of the algal species have a tendency to remove mercury from media by volatilization (De Filippis and Pallaghy 1976; Wilkinson et al. 1990; Devars et al. 2000). **Ben-Bassat and Mayer (1977)** found that *Chlorella* contain a reducing factor that converts Hg^{2+} in $HgCl_2$ to Hg^0. It is previously reported by De Filippis (1978) that volatilization of mercury by *Chlorella* sp. required an enzyme system, sulphydryl groups and NADPH/NADP as the energy source. The free metal ion concentration is regulated by complex interactions between metal ions, ligands. It is also regulated by pH of the solution (Sigg and Xue 1994; Mehta and Gaur 2005). **Solisio et al. (2017)** investigated the biosorption of Hg at pH 2-8 on dry biomass of *Chlorella vulgaris*. The maximum sorption was achieved at pH 5. FT-IR analysis confirmed that biosorption occurred via physisorption attributed to cation-exchange capacity of the biosorbent. Besides,

2.2.6 Lead

The cellular macromolecules and enzymes with appropriate functional groups or metal co-factors add their role in metal binding activity of algal cells (Mehta and Gaur 2005). Once the metal ions enter the cell, they precipitate or complex with intracellular compone00nts (Gadd 1988). **Edris et al. (2014)** reported the biosorption of cadmium and lead by algae *Chlorella vulgaris*. The higher initial ion concentration provides larger driving force to overcome mass transfer

resistance between solid and aqueous phase. About 90% of lead had adsorbed in first 20 minutes and afterwards, the process slowed down. This results in higher metal ion adsorption, which is in agreement with findings of prior studies (Bates et al. 1982; Chen et al. 2008; Riaz et al. 2009).

Hameed (2006) studied the effect of free live or dead cells of *Chlorella vulgaris* on removal of lead and compared with alginate immobilized algal cells and blank beads. The immobilized algae and the alginate blank beads significantly remove more lead as compared to free cells. Some studies have reported that immobilization causes increase in metal accumulation by biomasses (Aksu et al. 1998). Low amount of lead eluted from the free live and killed algal cells compared to immobilized algal beads or alginate blank beads indicates *Chlorella* bounds lead metal ion more strongly than immobilized cells (Hameed 2006). Haug (1967) found the affinity of alginates were increased with guluronic acid content for divalent cations such as Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, Ca^{2+}, etc.

2.2.7 Cadmium

The major sources of Cadmium (Cd) exposure to humans are industries, contaminated food and cigarettes smoking (IARC 1993; Paschal et al. 2000). Cadmium causes severe pulmonary and gastrointestinal irritation and may be fatal if inhaled or ingested (Baselt and Cravey 1995). Chronic exposure to cadmium induces a depressive effect on norepinephrine, serotonin, and acetylcholine (Singhal et al. 1976). **Shim et al. (2009)** investigated the effect of *Chlorella* on cadmium toxicity in rats. The accumulation of Cadmium in blood and other tissues was found to be low, whereas fecal and urine concentration was higher in rats fed with *Chlorella* diet in dose dependent manner compared to rats fed with Cd-diet only. From results, it suggested that *Chlorella* not only binds Cd ion but also associate prevent toxicity by decreasing its absorption to systemic circulation and increasing excretion through fecal and urine.

Ting et al. (1989) studied the effect of *Chlorella vulgaris* on cadmium and zinc accumulation and proposed two mechanisms: surface adsorption and membrane transport. The surface

adsorption does not involve any energy expenditure by cells and hence a small quantity of cadmium bonded independent of light and temperature. However, the rate of membrane transport had found to be dependent on cell energetics and metabolism as the exclusion of light limits transport at 25ºC.

3. Rationale of the Work

Presence of radioactive materials inside the body causes accumulation in the different organs of body. It is required to remove the radionuclide from GIT and systemic circulation by using surface active adsorbent or chelating agents. The decorporating agents can be used for prevention of radionuclides absorption from g.i.t to systemic circulation, hastening the elimination of absorbed radionuclides from blood stream or decorporation of radionuclides from their target organs. The compounds such as diethylene triamine pentaacetic acid (DTPA), D-Penicillamine, Calcium gluconate, Potassium Iodide and Prussian blue are the most widely used decorporating agent for removal of radionuclide and heavy metal ions. These agents are low therapeutic index and given only under medical supervision.

In this approach, the availability of a non-toxic decorporation agent for administration as soon as possible after a radiological event is critically important. Algae accumulate high concentrations of metals depending on their concentration in the external environment. The concentration factor for heavy metals varies greatly in different algal species (Sharma and Azeez, 1988). *Chlorella* (green algae) is consumed as dietary supplement. Dried *Chlorella* consist of about 45% protein, 20% fat, 20% carbohydrate, 5% fiber, and 10% minerals and vitamins. It reported that *Chlorella* is used in decorporation of heavy metals like Cadmium (Shim et al., 2009) and lead (Queiroz et al., 2003). Furthermore, another study reported *Chlorella* significantly enhances the elimination of radioactive strontium in rats (Ogawa et al., 2016). In concern of this the present study will envisaged to screen the binding and removal efficacy of pulverized *Chlorella* for radionuclide and/or heavy metal ions.

4. Aim and Objective

The aim of the present study is to evaluate the *Chlorella* as a potential drug for nuclear disaster management.

The objective of this study is to explore application of *Chlorella* for the internal decorporation of radionuclide. The specific objectives aimed in the present work are

- Evaluation of *in-vitro* binding/adsorption efficiency of *Chlorella*, for nonradioactive isotopes of nuclear fission produces radionuclides.
- Evaluation of *in-vitro* chelation effect of *Chlorella*, for nonradioactive isotopes of nuclear fission produces radionuclides.
- Evaluation of *in-vivo* removal efficacy of *Chlorella*, for nonradioactive isotopes of nuclear fission produces radionuclides in animal model and its toxicity studies.

5. Plan of Work

5.1 Evaluation of *in-vitro* binding/adsorption efficiency of *Chlorella*, for nonradioactive isotopes of nuclear fission produces radionuclides.

5.1.1 Drugs and Chemicals

Chlorella powder (Daesang Co. Ltd., Korea) and Nonradioactive metal ions like Mercury chloride, Strontium chloride, Cobalt chloride, Barium chloride, Thallium chloride and Cesium chloride will be procured from local supplier.

5.1.2 Adsorbate and adsorbent preparation

The stock solution of adsorbate (1000 mg/L) will be prepared by dissolving weighed quantity of the salt of heavy metal or radionuclides (Hg, Sr, Co, Ba, Tl and Cs) in distilled water. The *Chlorella* powder will be obtained commercially and used for the preparation of adsorbent.

5.1.3 Assessment of in-vitro binding efficiency of *Chlorella*

The present study will investigated for adsorption efficiency of *Chlorella* for different radionuclides metal ions in terms of weight of adsorbent (0.1-100 g/L), contact time (30-300 min), metal ion concentration (0.01-1 mg/ml) and influence of pH. The batch experiment was conducted to determine the optimum sorption of radionuclide metal ions in each parameter. For determination of effect of pH on binding efficiency of *Chlorella*, buffer of different pH range (pH 3-6) and physiological fluids like gastric (pH 1.2) and intestinal (pH 6.8) simulated fluid will be prepared and introduced a known quantity of *Chlorella* and metal ion.

Weighed quantity of *Chlorella* 1, 10, 50, 100, 500 and 1000 mg will be introduced in 10 ml of distilled water containing radionuclide metal ions. The solution containing *Chlorella* and metal ions will be incubated for different time point of 30, 60, 90, 120, 150 and 180 min with intermittent shaking. After each time point, the reaction mixture will be filtered through Whatman filter paper (0.45 μm) to remove solid particles and the filtrate will be analyzed by atomic absorption spectrophotometer for determination of metal ion content.

5.1.4 Adsorption Isotherm Model: Sorption capacity was analyzed by subjecting the data to various adsorption isotherm models like Langmuir and Freundlich models.

Langmuir model is represented by:

$1/q = 1/Q + 1/KQ \times 1/Ce$

The linear plot of specific adsorption (1/q) against the equilibrium concentration (1/Ce) shows that the adsorption obeys the Langmuir model. The constants Q and K relate to the energy of adsorption and maximum adsorption capacity.

Freundlich model is represented by:

$\text{Log } q_e = \log K_F + 1/n \log C$

Where q_e represents the amount adsorbed per amount of adsorbent at the equilibrium (mg/g), Ce represents the equilibrium concentration (mg/L), and K_F and n are constants that depend on the adsorbate and adsorbent

5.1.5 Scanning Electron Microscope (SEM) and FTIR

The surface morphology and binding mechanism of radionuclides on *Chlorella* will be studied by using scanning electron microscope (JEOL JSM-6610LV SEM). The *Chlorella* powder (10 g/l) will be incubated for 3 h with occasional shaking in solution containing metal ion (10 mg/ml). The *Chlorella* will be filtered and dried. The dried powder will be layered onto carbon tape and placed on a grid. This will be sputtered coated with Platinum. The structure and surface morphology of radionuclide metal ion treated *Chlorella* will be observed under SEM.

The dried *Chlorella* powder will be processed to record IR spectrum using FTIR Perkin Elmer Model no-1400. The spectra range 500 cm^{-1} to 4000cm^{-1} was used for scanning.

5.2 Evaluation of *in-vitro* chelation effect of *Chlorella*, for nonradioactive isotopes of nuclear fission produces radionuclides

The in-vitro chelation effect of *Chlorella* will be analyzed by determining the effect and contribution of several metal ions on Ca^{2+} measurement in Peripheral blood mononuclear cells (PBMCs) loaded with fura-2AM.

5.2.1 Drugs and Chemicals

Chlorella Powder (Daesang Co. Ltd., Korea), Fura-2/AM (Sigma-Aldrich), Non-radioactive metal ions (Co, Sr, Ba, Cs, Tl or Hg), RPMI-1640 Media, Histopaque®-1077, Fetal Bovine Serum and Hepes Buffer.

5.2.2 Preparation of Drug

The *Chlorella* powder will be extracted with Milli-Q water and filtered by using Whatman filter paper. The filtrate will be passed through 0.2 µm syringe filter. This dissolved part of *Chlorella* will be used for assessment of chelation effect.

5.2.3 Isolation of PBMCs

Peripheral blood mononuclear cells (PBMCs) are chiefly lymphocytes and monocytes. PBMCs will be separated from the whole blood by a density gradient centrifugation method using Histopaque®-1077. PBMCs will be suspended in RPMI 1640 media containing 10% fetal bovine serum, 100 unit/ml penicillin and 100 µg/ml streptomycin and placed at 37°C in a humidified 5% CO_2 atmosphere. Cells will be seeded at a density of 5×10^4 cells/cm^2 in a 96-well plate.

5.2.4 Measurement of fura-2 flourescence intensity

Cells will be seeded at a density of 5×10^4 cells/cm^2 in a 96-well plate. The Cells will be incubated with 5µM fura-2/AM at 37°C for 16-24h. After 24h incubation at 37°C, fura-2 fluorescence will be measured at 510 nm emission after excitation at 340 nm and 380 nm using a plate reader. The change in Ca^{2+} was reflected by the ratio of fluorescence intensities at 340 and 380 nm. To determine the changes in fura-2 fluorescence ratio induced by heavy metal or radionuclides, cells will be treated with mercury chloride, strontium chloride, cobalt chloride, barium chloride, cesium chloride or thallium chloride dissolved in distilled water. The *Chlorella* will be added 30 min after the stimulation with heavy metals to determine the contribution of endogenous and exogenous heavy metals on fura-2 fluorescence changes. The effect of different concentration of *Chlorella* on the fura-2 fluorescence ratio will be measured after 16-24h treatment with *Chlorella*.

5.2.5 Measurement of extra- and intracellular concentration of radionuclides

The treated PBMCs will be digested with 5-10% Nitric acid and centrifuged. The supernatant will be analysed for extra- and intracellular concentration of radionuclides using graphite furnace AAS

5.2.6 Fluorescence Microscopy

Labeled cells attached to micro-plate covered with coverslips will be studied under fluorescence microscope using excitation lights centered at wavelength 360 nm and emission 450 nm.

5.3 Evaluation of *in-vivo* removal efficacy of *Chlorella*, for nonradioactive isotopes of nuclear fission produces radionuclides in animal model and its toxicity studies

The *in-vivo* efficiency of *Chlorella* will be assessed only against the radionuclides which will be found to be effective in *in-vitro* studies.

5.3.1 Experimental Animals

Swiss albino mice (25-35g) of either sex will be used for the assessment in-vivo radionuclide removal efficiency of *Chlorella*. The animals will be acclimatized to standard laboratory conditions of temperature 22±3 °C with relative humidity 55±5% under 12h light:12h dark cycle. Animals will be provided with regular standard feed and drinking water *ad libitum*. All the protocols and experiments will be approved by the institutional animal ethical committee (IAEC) and conducted according to ethical guidelines provided by committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi.

5.3.2 Effect of *Chlorella* for internal decorporation of radionuclide

For each radionuclide the Swiss albino mice will divided into five groups (n=6). Non-radioactive isotopes (low or high dose, dissolved in distilled water) and *Chlorella* suspended in water will be administered once, orally during experimental period.

Experimental Design

The animals will be divided into different groups (n=6) and treated as following

- Non-radioactive isotopes (Hg, Sr, Co, Ba, Cs or Tl) in low or high doses
- *Chlorella* powder suspended in water in low medium or high doses.

All the doses were prepared in distilled water and administered orally. At the end of experiment blood, urine, faeces and organs will be collected and analysed for radionuclide metal ion content by using atomic absorption spectrophotometer (AAS).

Collection and Analysis of Blood:

At the end of the experiment, blood samples will be collected from each animal from retro-orbital plexus under anesthetic conditions and analyze for complete blood count (CBC). The remaining blood will be digested using 10% nitric acid and the supernatant will be separated and analysed for radionuclide metal ion content using AAS.

Collection and Analysis of Urine and Faeces:

In acute dose study, immediately after dosing the animal will keep separately in metabolic cages and, urine and faeces of 24h will be collected. In repeated dose study urine and faeces of 24h will be collected daily till the experimental period. The weighed quantity of urine and faecal samples will be digested using nitric acid and the supernatant will be separated and analysed for radionuclide metal ion content using AAS.

Collection and Analysis of Organs:

To determine the metal ion concentration in organs, the animals will be sacrificed at the end of the experiment. The organs viz., kidney, liver, spleen, heart, brain, stomach, intestine, colon, muscle and bone will be collected. The weighed quantity of organ will be digested with nitric acid and homogenized using bead beater homogenizer. The sample will be centrifuged and supernatant collected. The supernatant will be analysed for radionuclide metal ion content in different organs using AAS.

5.3.3 Toxicity studies

Oral toxicity study for *Chlorella* will be carried out as per Organization for Economic Co-operation and Development (OECD) guidelines (OECD guidelines, 2001).

5.4 Statistical Analysis

The results will be expressed as mean ± S.E.M. (n=6). The data will be analysed statistically using GraphPad Prism 5.00. Statistical analysis will be performed by one way ANOVA followed by Dunnett's Multiple Comparison Test. A value of $P<0.05$ will be considered significant in all cases.

6. Expected outcome

In the backdrop of the recent radiological accidents like the Fukushima tragedy, internal contamination with radionuclides due to ingestion, inhalation or via skin has become a serious threat. There are a number of drugs available for the removal of internalized radionuclides under

medical supervision. However, there is a pressing need to explore drug/nutraceuticals having significant potential to bind with radionuclides, which can be used in nuclear emergencies. In this context, the present study will explore the potential of microalgae *Chlorella* for the removal of radionuclides. *Chlorella* will be extensively studied as biosorbent for various radioisotopes in contrast to various factors like, pH, contact time, presence of other metal ions does affect the efficiency of *Chlorella*. However, all these parameters will be optimized to achieve the best possible binding efficacy. In addition to biosorption, pulverized *Chlorella* powder will offers an excellent option for the removal of internalized radioisotopes.

7. References

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